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(54) Title: USE OF THIAMPHENICOL FOR THE TREATMENT OF *CHLAMYDIA PNEUMONIAE* INFECTIONS

(57) Abstract: Use of thiamphenicol and derivatives thereof for the preparation of pharmaceutical compositions useful for the treatment of *Chlamydia pneumoniae* infections is described.

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
IT-368		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 01/ 03709	02/04/2001	11/04/2000
Applicant		
ZAMBON GROUP S.P.A.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

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2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

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USE OF THIAMPHENICOL FOR THE TREATMENT OF CHLAMYDIA PNEUMONIAE INFECTIONS

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[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/03709

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, WPI Data, PAJ, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 01378 A (BAUMGART KARL WILLIAM :BORODY THOMAS J (AU)) 13 January 2000 (2000-01-13) claims 1,8,9 ---	1-6
P,X	LOMBARDI ALESSANDRA ET AL: "Antimicrobial activity of thiamphenicol-glycinate-acetylcysteinate and other drugs against Chlamydia pneumoniae." ARZNEIMITTEL-FORSCHUNG, vol. 51, no. 3, 2001, pages 264-267, XP001019117 ISSN: 0004-4172 abstract --- -/--	1-6

[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
7 December 2001	17/12/2001

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	F BLASI: "Clinical featrus of chlamydia pneumoniae acute respiratory infection" CLINICAL MICROBIOLOGY AND INFECTION, vol. 1, no. 1, 1 March 1996 (1996-03-01), pages s14-s18, XP001037674 lONDON, GREAT BRITAIN the whole document	1-6

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 01/03709

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0001378 A	13-01-2000	AU 4591999 A	24-01-2000
		WO 0001378 A1	13-01-2000
		EP 1093363 A1	25-04-2001

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(54) Title: METHODS AND COMPOSITIONS FOR TREATMENT OF DISORDERS ASSOCIATED WITH CHLAMYDIAL AND SIMILAR BACTERIAL INFECTION

(57) Abstract

There are provided methods and pharmaceutical compositions for the treatment or prevention of conditions associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment or prevention. The methods of the invention comprise the administration to the patient of an effective amount of at least two different antibiotics or antimicrobial agents selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co–trimoxazole and oxazolidinones. Compositions of the invention comprise at least two antibiotics or antimicrobial agents selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co–trimoxazole and oxazolidinones.

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METHODS AND COMPOSITIONS FOR TREATMENT OF DISORDERS ASSOCIATED WITH CHLAMYDIAL AND SIMILAR BACTERIAL INFECTION

Technical Field

The invention relates to pharmaceutical compositions and methods for the treatment of vascular disease and other diseases either resulting from, aggravated by or associated with infection by *Chlamydia pneumoniae*, other *Chlamydia* species and similar susceptible microorganisms.

Background of the Invention

Vascular disease remains a major cause of morbidity and mortality worldwide. The development of atheromatous plaque within vessel walls followed by complications such as plaque rupture with activation of the clotting cascade and occlusion of the vessel resulting in infarction of distant tissue accounts for the majority of myocardial infarction, ischaemic stroke and other ischaemic tissue injury. Conventional therapy for vascular disease seeks to prevent or reverse clot formation or to reduce vascular disease risk factors such as dyslipidaemia or hypertension. *Chlamydia pneumoniae* is a recently described microorganism, which has been identified in atherosclerotic plaque and incriminated in vascular disease. It is an obligate intracellular pathogen that grows within macrophages and endothelial cells. Infection with *C. pneumoniae* is characterised by intracellular persistence following infection. Approximately 50% of the population are seropositive for *C. pneumoniae* in adult life and most persons acquire the infection by the respiratory route. Not all persons infected with *C. pneumoniae* develop vascular disease, however. Recovery rates of the microorganism have ranged between 20 to 60% of sites of atherosclerotic tissue and the organism has not been recovered from normal vascular tissue. Animal models have been developed in which infection with *C. pneumoniae* is followed by the development of atherosclerotic plaque. To date, however, Koch's postulates have not been fulfilled for *C. pneumoniae* in human atherosclerotic vascular disease, and this is in part a consequence of the serious nature of challenge testing as well as the fact that the organism is an obligate intracellular pathogen. Two limited therapeutic studies have been published in which there appears to be a benefit after monotherapy with a macrolide antibiotic. In one of these studies (Gupta S, et al., *Circulation* 1997, **96**, 404-407) azithromycin was used, and the benefit was not sustained after initial therapy. In the other study (Gurfinkel E, et al., *Lancet* 1997, **350**, 404-407) roxithromycin was used as sole therapy and in limited numbers a benefit was described, although prolonged

follow-up has not yet been reported. In another study (Sinisalo J, et al., *J. Antimicrob. Chemother.* 1998, **41**, 85-92) tetracycline antibiotics were used as monotherapy and no clinical benefit was discerned.

In the case of "difficult to eradicate" intracellular pathogens, widespread use of single antibiotic regimes has serious potential adverse consequences for the population at large as well as for individuals who may develop resistant infections. Important examples of these problems in other areas of clinical practice include tuberculosis, leprosy and *Helicobacter pylori* infections. A further feature of largely intracellular infections such as those in which combination regimes have come to be used relates to the concept of "suppression" versus "eradication" following treatment. Although a course of macrolides in the treatment of *C. pneumoniae* can result in seemingly measurable early improvement clinically, the patients remain at risk of developing a recrudescence of the intracellular infection which has been merely suppressed rather than eradicated. With regrowth of the bacteria, the disease returns and the likelihood of response to repeated therapy is diminished, with the spectre of antimicrobial resistance. Furthermore, widespread use of single antibiotic regimes may result in greater resistance amongst *C. pneumoniae* and other important human pathogens than those being treated. Until now it has not been realised that antibiotic monotherapy which could result in a transient improvement in clinical parameters, was actually an indication of the suppression of the bacterial growth, with probable entry of the bacteria into a more intracellular yet chronic phase of infection.

There is therefore a need for methods of treating conditions associated with infection by *C. pneumoniae* and similar susceptible microorganisms which treat the initial infection so as to prevent the chronic phase of infection with its consequences of ongoing disease and heightened bacterial resistance.

The present inventors have found that a multi-drug therapy regimen is well tolerated and has a superior clinical efficacy in resolution of infections caused by *Chlamydia* species and similar susceptible microorganisms, and particularly *C. pneumoniae*, compared to monotherapy. Indeed, the method of treatment of the present invention is more likely to cure the infection rather than simply suppress it and is more likely to prevent the development of resistant isolates.

The use of multiple antibiotics for *C. pneumoniae* infection had not been studied prior to the date of the present invention. Experts in the field who have initiated clinical trials before and after the date of the present invention have only used single agent regimes. Prior to the date of the present invention, it had not been considered necessary or desirable to use multiple antibiotic regimes for the treatment of *Chlamydia pneumoniae*,

other *Chlamydia* species and similar susceptible microorganisms. Furthermore, although there have been therapies for such infections in the past which have been considered adequate in the past, the present inventors have observed that with the passage of time there has been a change of bacterial susceptibility in communities towards more "difficult-to-cure" infections requiring the invention and development of more aggressive, yet safe, therapies. It is an object of the present invention to provide one such improved therapy.

Summary of the Invention

Accordingly, in a first embodiment the present invention provides a method for the treatment or prevention of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment or prevention, the method comprising the administration to the patient of an effective amount of at least two different antibiotics or antimicrobial agents selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones.

Throughout the present specification, "similar susceptible microorganisms" are defined as including other difficult to culture, "atypical" agents such as *Mycoplasma* species, *Listeria* species, *Bartonella* species, and the aetiologic agents of Leptospirosis and Q fever.

The present invention also provides the use of at least two different antibiotics or antimicrobial agents selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones for the manufacture of a medicament for the treatment or prevention of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment.

The present invention further provides at least two different antibiotics or antimicrobial agents selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones, when used for the treatment or prevention of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment.

In a second embodiment, the invention provides a pharmaceutical composition for the treatment or prevention of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment or prevention, the pharmaceutical composition comprising at least two different antibiotics or

antimicrobial agents selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones.

In a third embodiment, the invention provides a pharmaceutical composition comprising a first antibiotic or antimicrobial agent and at least a second antibiotic or antimicrobial agent wherein at least one of said antibiotics or antimicrobial agents is provided with a pharmaceutically acceptable coating, said antibiotics or antimicrobial agents being selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones.

In a fourth embodiment, the invention provides a process for preparing a pharmaceutical composition, the process comprising providing a first antibiotic or antimicrobial agent with a first pharmaceutically acceptable coating and providing at least a second antibiotic or antimicrobial agent, optionally with a second pharmaceutically acceptable coating, and incorporating the coated first antibiotic or antimicrobial agent and the optionally coated second antibiotic or antimicrobial agent into a single dosage form, said antibiotics or antimicrobial agents being selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones.

The invention also provides a pharmaceutical composition when prepared by the process of the fourth embodiment.

In further embodiments, the invention provides a method of the first embodiment which further includes the administration of a third, or a third and a fourth, or a third, a fourth and one or more further different antimicrobial agents or antibiotics. Similarly, the invention provides a pharmaceutical composition of the second embodiment which includes a third, or a third and a fourth, or a third, a fourth and one or more further different antimicrobial agents or antibiotics.

Detailed Description of the Invention

Throughout this specification, the word "comprising" is to be understood to mean "including principally, but not necessarily solely". Variants such as "comprise" or "comprises" are to be understood to have corresponding meanings.

The present invention provides methods and pharmaceutical compositions for treating patients either with, or at risk of, vascular disease following infection with *C. pneumoniae* and similar susceptible microorganisms. The methods of the invention result in a cure of the infection and reversal of the clinical condition. The invention further

provides combinations of antimicrobial agents effective against intracellular pathogens including *C. pneumoniae* and other *Chlamydia* species. The invention therefore provides methods and pharmaceutical compositions for the treatment not only of vascular complications of *Chlamydia* infections but also disorders resulting from or aggravated by such infections. Such disorders include asthma, chronic obstructive lung disease, dementia, urinary and gynaecologic mucosal Chlamydial infections.

Thus, the invention provides methods and pharmaceutical compositions for the eradication of persistent *Chlamydia* infections in individuals with disorders that are a manifestation of or contributed to by said infection. These disorders include atherosclerotic vascular disease affecting coronary arteries, aorta, carotid arteries and other arteries including renovascular and glomerular disease, aortic vascular disease, peripheral vascular disease, carotid or cerebrovascular disease, atrial fibrillation and other cardiac arrhythmias, myocardial infarction, unstable or stable angina, valvular heart disease, cardiomyopathy, myocarditis and vasculitis; upper or lower respiratory tract infection; pneumonia; asthma; chronic airflow limitation; sarcoidosis; lung cancer; granulomatous hepatitis; dementia and gynaecologic and urologic mucosal infections.

Further, the methods and pharmaceutical compositions of the invention, in addition to having application for Chlamydial infections, have utility for clinical syndromes that result from infection by mycoplasma, Bartonella, Leptospirosis and Q fever.

In one broad form, the present invention relates to a method of treating patients with a previous or current infection with *C. pneumoniae* and similar susceptible microorganisms by administering a combination of at least one antibiotic or antimicrobial agent and at least a second antibiotic or antimicrobial agent, the first and second antimicrobial agents each being selected from the following classes of antibiotics or antimicrobial agents: tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones. A major application of the present invention is for the treatment of patients with, or at risk of, vascular disease following infection with *C. pneumoniae* or similar susceptible microorganisms. However, as disclosed herein above, the invention includes treatment or prevention of other disorders in which *C. pneumoniae* or similar susceptible microorganisms have an aetiological role.

In the methods and pharmaceutical compositions of the invention, the first antibiotic, the second antibiotic and any additional antibiotics are preferably, but not necessarily, selected from different classes of antibiotics as identified herein above. For example, the antibiotics used in a method of the present invention are preferably each selected from different classes wherein the classes are tetracyclines, macrolides,

quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones. Similarly, the antibiotics included in a pharmaceutical composition of the present invention are preferably each selected from different classes selected from tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones.

More preferably in the methods and pharmaceutical compositions of the invention the first antibiotic is selected from a macrolide antibiotic and the second antibiotic is selected from tetracyclines and quinolones. Thus, in one form of the second embodiment, the invention provides a pharmaceutical composition for the treatment of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment, the composition comprising a first antibiotic which is a macrolide antibiotic and a second antibiotic or antimicrobial agent which is selected from tetracyclines and quinolones.

More preferably when a three-drug regime is selected for a method of the present invention, then three antibiotics, each being from the following different classes, are selected, where the classes of antibiotics are tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones.

In another form of the second embodiment, the invention provides a pharmaceutical composition for the treatment of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment, the composition comprising at least two different antibiotics or antimicrobial agents selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, sulfonamides, co-trimoxazole and oxazolidinones and not including a rifamycin.

In still another form of the second embodiment, the invention provides a pharmaceutical composition for the treatment of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment, the composition comprising azithromycin, rifampicin and doxycycline or a quinolone.

In yet another form of the second embodiment, the invention provides a pharmaceutical composition for the treatment of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment, the composition comprising clarithromycin, rifampicin, and doxycycline.

In a further form of the second embodiment, the invention provides a pharmaceutical composition for the treatment of a condition associated with infection by

Chlamydia species or similar susceptible microorganisms in a patient in need of such treatment, the composition comprising roxithromycin, ofloxacin and rifampicin.

In the pharmaceutical composition of the third embodiment, preferably the second antibiotic or antimicrobial agent is provided with a pharmaceutically acceptable coating.

In one form of the pharmaceutical composition of the third embodiment, the coating or coatings are adapted to cause the first and second antibiotics or antimicrobial agents to be released in first and second environments in the gastrointestinal tract of a patient to whom the composition is administered, wherein an effective amount of the first antibiotic or antimicrobial agent is capable of being absorbed into the bloodstream of the patient from the first environment and an effective amount of the second antibiotic or antimicrobial agent is capable of being absorbed into the bloodstream of the patient from the second environment.

In another form of the pharmaceutical composition of the third embodiment, the composition further comprises a third antibiotic or antimicrobial agent provided with a third pharmaceutically acceptable coating. Typically, the third coating is adapted to cause the third antibiotic or antimicrobial agent to be released in a third environment in the gastrointestinal tract of a patient to whom the composition is administered, wherein an effective amount of the third antibiotic or antimicrobial agent is capable of being absorbed into the bloodstream of the patient from the third environment.

Preferably, in this form of the pharmaceutical composition of the third embodiment, the first antibiotic or antimicrobial agent is a macrolide, typically an azalide or ketolide, more typically azithromycin, clarithromycin or roxithromycin, the second antibiotic or antimicrobial agent is a rifamycin, typically rifampicin, and the third antibiotic or antimicrobial agent is a tetracycline (typically doxycycline), or a quinolone, typically but not restricted to ofloxacin.

Alternatively, in this form of the pharmaceutical composition of the third embodiment, the first, second and third antibiotics or antimicrobial agents are selected from the group consisting of clarithromycin, rifabutin, rifampicin, azithromycin, roxithromycin, amikacin, clofazimine, ethambutol, ofloxacin, ciprofloxacin and oxazolidinone. More typically in this form the first, second and third antibiotics or antimicrobial agents are clarithromycin, rifabutin and clofazimine. In such a composition, the amount of clarithromycin is typically from 200-300mg, more typically about 250mg, the amount of rifabutin is typically from 50-250 mg, more typically about 150mg, and the amount of clofazimine is typically from 10-150mg, more typically about 50mg.

In one particularly preferred method in accordance with the invention there are administered azithromycin in an amount of 500 mg per day, rifampicin in an amount of 300 mg per day, and doxycycline in an amount of 100 mg per day and/or a quinolone; the administration being daily for four weeks.

In another particularly preferred method in accordance with the invention there are administered clarithromycin 500 mg per day, rifampicin 300 mg per day, and doxycycline 100 mg per day; the administration being orally, daily for four weeks.

In a further particularly preferred method in accordance with the invention there are administered roxithromycin 300 mg per day, ofloxacin 400 mg per day, and rifampicin 300 mg per day; the administration being orally, daily for four weeks.

One preferred pharmaceutical composition in accordance with the invention includes azithromycin in an amount of 250 mg, rifampicin in an amount of 150 mg, and doxycycline in an amount of 50 mg.

Another preferred pharmaceutical composition in accordance with the invention includes clarithromycin in an amount of 250 mg, rifampicin in an amount of 150 mg, and doxycycline in an amount of 100 mg.

A further preferred pharmaceutical composition in accordance with the invention includes roxithromycin in an amount of 150 mg, ofloxacin in an amount of 200 mg, and rifampicin in an amount of 150 mg.

Examples of tetracyclines suitable for use in the methods and pharmaceutical compositions of the present invention include tetracycline, oxytetracycline, doxycycline, demeclocycline, chlortetracycline, methacycline and minocycline. Examples of macrolide antimicrobial agents suitable for use in the methods and pharmaceutical compositions of the present invention include erythromycin (including various forms occurring as base, stearate, ethyl succinate, lactobionate, gluceptate and estolate), clarithromycin, azithromycin, roxithromycin, spiramycin, oleandomycin, triacetyloleandomycin, josamycin, kitsamycin, midecamycin, miocamycin, rokitamycin, rosarimycin, flurithromycin dithromycin as well as other azalides and ketolide antibiotics. Examples of quinolone antibiotics suitable for use in the methods and pharmaceutical compositions of the present invention include nalidixic acid, oxolinic acid, norfloxacin, pefloxacin, amifloxacin, ofloxacin, ciprofloxacin, enoxacin, lomefloxacin, fleroxacin, temafloxacin, sparfloxacin, tosufloxacin, clinafloxacin, cinoxacin, trovafloxacin, levofloxacin, nadifloxacin and rufloxacin. Examples of the rifamycin class of antimicrobial agents suitable for use in the methods and pharmaceutical compositions of the present invention include rifampicin, rifabutin and rifapentin. Examples of sulfonamides suitable for use in

the methods and pharmaceutical compositions of the present invention include sulfisoxazole, sulfamethoxazole, sulfadiazine, sulfadoxine, sulfasalazine, sulfaphenazole, dapsone, and sulfacytidine, Other classes of antibiotics suitable for use in the methods and pharmaceutical compositions of the present invention include co-trimoxazole and oxazolidinones. Examples of oxazolidinones include linezolid and oxazolidinone.

The doses of the antibiotics or antimicrobial agents used in this invention are in accordance with their generally known and established safe dosage ranges when they are used in monotherapy for the treatment of other conditions.

Such dosages for antibiotics or antimicrobial agents are well known to medical practitioners and range from 0.0005 to 50 g per day, depending on the agent. Safe dosages of antibiotics and antimicrobial agents for use in the methods of the present invention are described, for example, in Martindale, The Extra Pharmacopoeia, Thirty-first Edition (The Royal Pharmaceutical Society, London, 1996). Administration may be by oral, intravenous, intra-arterial, intramuscular, inhalation, topical and subcutaneous routes. Typically, administration is by the oral route. Administration of each of the antibiotics or antimicrobial agents may be in a single daily dose, or in two or more doses per day. Typically the antibiotics or antimicrobial agents are administered to the patient essentially simultaneously, but they need not be.

Pharmaceutical compositions of the present invention typically include the active substances in amounts of from 10% to 100% of the respective daily doses, more typically from 20% to 50% of the daily doses.

Typically, in the methods of the present invention, the combination of first and second antibiotics, and optionally one or more additional antibiotics or antimicrobial agents is administered for between 1 and 28 days. However, the treatment may be continued, as may be indicated in certain clinical circumstances, especially in previously treated patients, for up to 6 months. More prolonged therapy may be indicated in patients who are unable to have initial therapy at adequate doses because of intolerance to any of the antimicrobial agents in the composition, in patients who experience a relapse of their clinical condition, or in patients who have evidence of ongoing inflammation or ongoing inflammatory markers.

Treatment of conditions associated with *C. pneumoniae* is typically continued until the following conditions are established in the patient: peripheral blood PCR detection of *C. pneumoniae* reduced to undetectable levels; normalisation of inflammatory markers including ESR, CRP and serum amyloid A protein; reduction in *C. pneumoniae* IgA titre and reduction in *C. pneumoniae* IgG titre; disappearance of identifiable *C. pneumoniae* from

bowel or bronchial biopsies by antigen detection methods or molecular methods; disappearance of *C pneumoniae* from sputum or stool tests by antigen detection methods or molecular methods. Similar diagnostic indicators are utilised for monitoring the effectiveness of a method of treatment of the invention for similar susceptible microorganisms to *C. pneumoniae*.

Preferably the initial treatment is followed by maintenance therapy with intermittent dosing of combined antibiotics on a periodic basis which may range from an additional day or more days per month for additional months or years. In some patients, dormant or persistent organisms may require treatment regimes to be extended for many years.

In one preferred form, the invention provides pharmaceutical compositions presented in a format suitable for specific clinical circumstances. For example, for patients with vascular disease, atypical pneumonia syndromes or pelvic inflammatory disease in particular, these special methods of administration are recommended. As described in more detail below, pharmaceutical compositions of the invention may be provided as single dosage forms which release the active substances into the bloodstream of a patient to whom they are administered in a controlled manner so as to prevent a sudden increase in the plasma concentration of the agents as would occur if they were adminstered, for example, as separate tablets. Since patients with conditions as exemplified above are often unwell and are often on other therapy, it is envisaged that these enhancements to delivery will reduce adverse experiences by patients including gastrointestinal tract intolerance and will minimise interactions with other concomitant therapy. Combined packaging as described below should also enhance compliance and therefore clinical outcomes.

A pharmaceutical composition of the second or third embodiment may be provided in sequential packaging for each antimicrobial agent or in daily compliance-enhancing packaging in which antimicrobial agents are formulated within a common capsule or tablet. In a further form, a sequence package of medications is provided for intravenous or intramuscular use followed by oral use.

In one form of the method of the first embodiment, the method further comprises the administration of one or more other medications used in the management of coronary and other vascular disease.

In another form of the method of the first embodiment, the method further comprises the administration of one or more other medications that enhance host defence mechanisms important in the eradication of intracellular pathogens.

Preferably the method of the first embodiment, when used for the treatment of patients with cardiovascular disease, further includes the administration of one or more additional agents selected from selective and non-selective cyclooxygenase inhibitors such as aspirin; other antiplatelet drugs such as ticlodipine or clopidogrel; betablockers; antiarrhythmics; calcium channel blockers, other anticoagulant drugs such as coumadin or heparin; nitrate medicines and HMG-Coareductase inhibitors. Examples of betablockers include inderal, metoprolol and atenolol; examples of antiarrhythmics include amiodarone, lignocaine, sotalol and flecanide; examples of calcium channel blockers include amlodipine, diltiazem and verapamil; examples of nitrate medicines include isosorbide mononitrate and nitroglycerin.

The methods of treatment of the first embodiment, when used for the treatment of patients who may have persistent *Chlamydia* infection, may further include the administration of one or more immune response modifiers selected from cytokines, including interleukin 1, interleukin 2, interleukin 3, interleukin 4, interleukin 5, interleukin 6, interleukin 7, interleukin 8, interleukin 9, interleukin 10, interleukin 11, interleukin 12, interleukin 13, interleukin 14, interleukin 15, interleukin 16, interleukin 17, interleukin 18, interleukin 19, interleukin 20; colony stimulating factors including G-CSF, GM-CSF; tumour necrosis factors alpha and beta; interferon alpha, beta and gamma; peptides which bind to macrophage and lymphocyte surface receptors; glycoproteins which mimic cytokines; and other mediator molecules.

The methods of treatment of the first embodiment may further include the administration of one or more other drugs with immunosuppressive activity when active inflammation or inappropriately biased and deleterious host immune responses are present. Examples of suitable drugs with immunosuppressive activity include prednisone and related steroids, azathioprine, mofetil mycofenolate and related purine antagonists, cyclophosphamide and related alkylating agents, methotrexate and related folate antagonists, thalidomide, chloroquine and related antimalarial compounds, levamisole, cyclosporin A and similar immunosuppressive agents including rapamycin and FK506.

Similarly, the pharmaceutical compositions of the second and third embodiments may further include one or more other medications used in the management of coronary and other vascular disease and/or one or more other medications that enhance host defence mechanisms important in the eradication of intracellular pathogens, such medications being as further described herein above.

Dosages of further agents, such as those exemplified herein above, when used in the methods of the present invention, are in accordance with their generally known and

established safe dosage ranges. Such dosages are well known to medical practitioners and are described, for example, in Martindale, The Extra Pharmacopoeia, Thirty-first Edition (The Royal Pharmaceutical Society, London, 1996).

In the methods of the invention, each of the co-administered antibiotics or antimicrobial agents may be administered to and ingested by the patient as separate medications, for example in the form of separate tablets, capsules or sachets, or as separate intravenously administered agents. Such tablets or capsules may be packaged and administered to the patient, for example, in a compliance-enhancing package of separate containers. Alternatively, separate tablets, capsules, etc, may be packaged in blister packs designed to guide the patient to compliance with the dosing protocol. For example, where a method of the invention involves the administration of three different medications a blister pack may be constructed to house the three different medications on the blister pack in such a manner as to direct the patient to a morning dose of each of the three medications, a midday dose of two of the medications, and an evening dose of the three medications again. In such an arrangement, each blister pack strip of medications could constitute a day's therapy, thereby enhancing the likelihood of patient compliance. Other variations of the arrangement described, according to the desired dosage protocol, will readily suggest themselves.

Alternatively, to further simplify administration of the medications to the patient and the patient's compliance with the dosage protocol, the combination of medications may be provided as microparticles or microgranules, typically micro-encapsulated, and a predetermined mass of each drug, preferably in its micro-encapsulated form, may be included in a single capsule or tablet. Alternatively, the medications may be formulated into separate tablets or capsules which are then incorporated into a single larger tablet or capsule as the final dosage form. In this manner the combination of two, three or more antimicrobial agents described above optionally together with one or more other medications as described above may be combined into a single dosage form to simplify the medication process and to deliver special dose combinations not currently available in the size tablets and capsules currently on the market.

Pharmaceutical compositions of the second or third embodiments may include one or more pharmaceutically acceptable excipients, adjuvants, diluents or carriers which are generally known in the art.

Pharmaceutical compositions of the second embodiment or for administration in a method of the first embodiment may be prepared by means known in the art for the preparation of pharmaceutical compositions including blending, grinding, homogenising,

suspending, dissolving, emulsifying, dispersing and where appropriate, mixing of the active agents, optionally together with one or more excipients, diluents, carriers and adjuvants.

For oral administration, a pharmaceutical composition of the second embodiment may be in the form of tablets, lozenges, pills, troches, capsules, elixirs, powders, including lyophilised powders, solutions, granules, suspensions, emulsions, syrups and tinctures. Slow-release, or delayed-release, forms may also be prepared, for example in the form of coated particles, multi-layer tablets or microgranules.

Solid forms for oral administration may contain pharmaceutically acceptable binders, sweeteners, disintegrating agents, diluents, flavourings, coating agents, preservatives, lubricants and/or time delay agents. Suitable binders include gum acacia, gelatin, corn starch, gum tragacanth, sodium alginate, carboxymethylcellulose or polyethylene glycol. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable diluents include lactose, sorbitol, mannitol, dextrose, kaolin, cellulose, calcium carbonate, calcium silicate or dicalcium phosphate. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

Liquid forms for oral administration may contain, in addition to the active agents, a liquid carrier. Suitable liquid carriers include water, oils such as olive oil, peanut oil, sesame oil, sunflower oil, safflower oil, arachis oil, coconut oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides or mixtures thereof.

Suspensions for oral administration may further include dispersing agents and/or suspending agents. Suitable suspending agents include sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, poly-vinyl-pyrrolidone, sodium alginate or cetyl alcohol. Suitable dispersing agents include lecithin, polyoxyethylene esters of fatty acids such as stearic acid, polyoxyethylene sorbitol mono- or di-oleate, -stearate or -laurate, polyoxyethylene sorbitan mono- or di-oleate, -stearate or -laurate and the like.

Emulsions for oral administration may further include one or more emulsifying agents. Suitable emulsifying agents include dispersing agents as exemplified above or natural gums such as gum acacia or gum tragacanth.

In the pharmaceutical compositions of the third embodiment, coatings are applied to the medications preferably to deliver the antimicrobial agents differentially to different regions of the gastrointestinal tract. The coatings may be applied, for example, to tablets of the medications which are then incorporated into a single dosage form such as a tablet or capsule. As another possibility the coatings may be applied in a micro-encapsulation process and the microencapsulated medications formulated into tablets or capsules so as to provide all of the microencapsulated medications in a single dosage form. For example, the antimicrobial agents may be coated, for instance by microencapsulation, so that they are released at different rates in the gastric lumen, the distal duodenum and beyond – so enhancing absorption and reducing cross-reaction between the medications. Typically, in this form, an antibiotic or antimicrobial agent is provided with a coating which maximises its release in a part of the gastrointestinal tract in which it is most effectively absorbed, and which minimises its release in other parts of the gastrointestinal tract.

For example, in a composition containing azithromycin, rifampicin and doxycycline, the azithromycin may be microencapsulated so as to dissolve in the gastric acidic environment while the other two actives are microencapsulated so as to be substantially undissolved in that environment, the doxycycline being coated so that it is released in the conditions within the second part of the duodenum, and the rifampicin being coated so that it is released more distally. In such a way the agents are released into environments in which they are readily absorbed and the possibility of them cross-reacting is minimised.

As a further possibility, a pharmaceutical composition of the third embodiment may be provided as a multilayer dosage form, in which the antibiotics or antimicrobial agents are provided in separate layers, or as a core and one or more separate layers, the active substances being separated by at least one coating so that an active substance provided in an outer layer is released first into the bloodstream of a patient in an appropriate region of the gastrointestinal tract, and one or more other antibiotics or antimicrobial agents in one or more inner layers are released subsequently, when the first active has been substantially released and a coating which separates it from the other active or actives has dissolved or been eroded.

The regions of the gastrointestinal tract in which antibiotics or antimicrobial agents used in the methods or compositions of the present invention are most effectively

absorbed are generally known in the art. Similarly, suitable coatings for pharmaceutically active substances to delay release of the substances until they reach predetermined environments within the gastrointestinal tract are generally known in the art, as are techniques for microencapsulation with such materials. Examples of references describing such coatings and techniques are Kirk-Othmer's Encylopedia of Chemical Technology, Fourth Edition, Volume 7, pp 274-300 (Wiley-Interscience, 1993) and references cited therein; Remington's Pharmaceutical Sciences, 18th Edition, Chapters 90 and 91 (Mack Publishing Company, Easton, Pennsylvania, 1990) and references cited therein; Australian Patent numbers 601974 and 603568 and references cited therein; and United States Patent numbers 5914132, 5910322 and 588550, and references cited therein. Other relevant references include the following:

Ranade, V.V., *J. Clin. Pharmacol.*, 1991, **31**, 2-16;

Smart, J.D., et al., *J. Pharm. Pharmacol.*, 1984, **36**, 295;

Hovgaard, L. and Brondsted, H., *Crit. Rev. Ther. Drug Carrier Syst.*, 1996 **13**, 185-223; and

Leopold, C.S., *Pharm. Sci. Technol. Today*, 1999, **2**, 197-204.

The disclosures of each of the references cited above are incorporated herein by reference.

Examples of coatings for targeting release of a pharmaceutical substance in the stomach include modified celluloses such as hydroxymethyl cellulose and hydroxypropyl cellulose, polysaccharide gums, tragacanth gum, sodium carboxymethylcellulose, chitosan, acrylates and methacrylates. Where it is desired for an antibiotic or antimicrobial agent of a pharmaceutical composition of the invention to be released in the stomach, the antibiotic or antimicrobial agent need not be provided with a coating. Coating for targeting release of an antibiotic or antimicrobial agent of a pharmaceutical composition of the invention in the stomach may be, for instance, be polymers available from Röhm GmbH, Germany, and sold under the Trade Marks Eudragit E, Eudragit RL and Eudragit RS.

Examples of coatings for targeting release of a pharmaceutical substance in the lower intestine include enteric coatings such as methacrylic acid copolymers, cellulose acetate phthalate, cellulose acetate succinate and styrol maleic copolymers (Agyilirah, G.A., et al., in Polymers for Controlled Drug Delivery, pp 39-66; Tarcha, P.J. ed., CRC Press, Boca Raton (1991)).

Examples of coatings for targeting release of a pharmaceutical substance in the colon include pH resistant polymeric coatings such as Eudragit L and Eudragit S and

bioerodable polymers such as shellac, ethyl cellulose, cellulose acetate phthalate and modified cellulose phthalates such as hydroxypropyl methylcellulose phthalate, cellulose acetate trimellitate, poly(vinyl acetate/vinyl alcohol) phthalate and cellulose acetoacetate mixed esters (United States Patent no 5811121).

Coatings may be applied to the active substances from solution in organic solvents or aqueous organic solvents. The coatings my include additives such as plasticisers, for example phthalic acid esters such as dibutyl phthalate, triacetin, fatty alcohols such as cetyl alcohol, citric acid esters, dibutyl succinate and the like; particulate dispersants such as talc and titanium dioxide; and colorants such as metal oxides or dyes. Solvents which may be used include methanol, ethanol, isopropanol, acetone, dichloromethane, diethyl ether, ethyl acetate and mixtures thereof. The choice of solvent is determined primarily by the solubility of the polymer and ease of evaporation of the solvent. Selection and amount of solvent, optional plasticiser, optional inert solid particulate and process of coating is made based upon the specific coating material used according to criteria known to those skilled in the relevant art. Coating methods are known to persons of ordinary skill in the relevant art and may utilise equipment such as fluidised beds, perforated pans, and spray equipment.

Examples

Example 1: Treatment of patients with coronary heart disease

Ten patients, aged 48-75, had had myocardial infarction and received acute therapy with thrombolytic agents. They subsequently proceeded to coronary angiography and subsequently stenting or coronary artery bypass surgery. Baseline cholesterol values were relatively low at less than 5.5 mmol/L. Despite good conventional management each patient developed recurrent angina within 12 months of revascularisation, documented by exercise Sestamibi testing. Serological testing by microimmunofluorescence assays and ELISAs confirmed "persistent" infection with *C. pneumoniae* with elevated IgA and IgG antibodies. The patients were treated with a combination of rifampicin 300 mg bd, doxycycline 100 mg twice daily as well as roxithromycin 150 mg twice daily for one month. Symptoms of ischaemic heart disease diminished following therapy and progress exercise Sestamibi studies confirmed improvement. Eighteen months later, no patients have had recurrence of their disease or progression of their disease. Assays of peripheral blood mononuclear cells showed a reduction in detectable *C. pneumoniae* DNA (by PCR) after therapy and specific IgA antibodies declined.

Example 2: Treatment of patients with atypical pneumonia and atrial fibrillation.

Three patients, aged 40-72, had been admitted to hospital with fever, cough and dyspnoea. They had clinical and radiological signs of bilateral pneumonia and evidence of significant hypoxia on arterial blood gas studies. In each patient, the pneumonia was investigated by acute and convalescent phase serology and found to be due to *C. pneumoniae*. All patients had recent onset atrial fibrillation. The patients received treatment with doxycycline 100 mg bd and azithromycin 500 mg twice daily for three weeks. In each patient, atrial fibrillation resolved within one week and pneumonia resolved within three weeks. One year later, no patient has had a recurrence of atrial fibrillation.

Example 3: Treatment of patients with vascular disease, *C. pneumoniae* infection and cancer

One patient, a 68 year old male had had bilateral carotid endarterectomies. Two years later he developed common bile duct obstruction. One year later he was diagnosed with cancer of the head of pancreas with hepatic metastases. He had a palliative small bowel anastomosis. He developed angina. He was found to have persisting IgA and IgG antibodies to *C. pneumoniae* by microimmunofluorescence testing. He declined cytotoxic chemotherapy for his pancreas cancer. He began therapy with 3 million units of interleukin-2 (subcutaneous 4 days every month) killed *Mycobacterium w* (10^7 organisms sc) and combined antibiotics including doxycycline 100 mg daily (three weeks) and roxithromycin 150 mg bd (three weeks). The metastatic lesions of pancreatic cancer regressed by more than 80% after three months of therapy. His CA19-9 returned to an undetectable level. Circulating CD4 T cells had not expressed TNF or gamma-interferon prior to cytokine therapy were found to have cytoplasmic expression of TNF and gamma-interferon three months after cytokine therapy. His angina ceased without ECG changes and did not recur. He remains well three years later, without angina, with no evidence of vascular disease and has no evidence of metastases on PET scans. IgA *C. pneumoniae* serology is negative.

Example 4. Pharmaceutical composition

The following is illustrative of a pharmaceutical composition in accordance with the invention.

Coating of doxycycline

A solution of Eudragit L100-55 (100 parts by weight) and dibutyl phthalate (20 parts by weight) is prepared in isopropanol:acetone:water (37:9:1 by weight; 1000 parts by weight) and micronised talc (40 parts by weight) is suspended in the solution. The solution is

coated onto a finely pulverised commercial preparation of doxycycline including lactose as inert (200 parts by weight) in a perforated pan coater maintaining an outlet air/bed temperature of about 30°C.

Coating of rifampicin

A solution of hydroxypropyl methylcellulose phthalate (100 parts by weight) and cetyl alcohol (5 parts by weight) in acetone:ethanol (2.5:1 by weight, 1300 parts by weight) is sprayed onto a finely pulverised commercial preparation of rifampicin containing lactose as inert (200 parts by weight) in a fluidised bed apparatus with spray guns placed above the bed.

Preparation of pharmaceutical composition in dosage form

Azithromycin, coated rifampicin and coated doxycycline in proportions of 5:3:1 by weight of active substances are blended and portions of the blend are encapsulated into gelatin capsules each containing 125mg azithromycin, 75mg rifampicin and 25mg doxycycline.

CLAIMS

1. A method for the treatment or prevention of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment or prevention, the method comprising the administration to the patient of an effective amount of at least two different antibiotics or antimicrobial agents selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones.

2. A method according to claim 1 which further includes the administration of a third, or a third and a fourth, or a third, a fourth and one or more further different antimicrobial agents or antibiotics.

3. A method according to claim 1 comprising the administration of at least three different antibiotics, each antibiotic being from a different class, selected from tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones.

4. A method according to claim 1 wherein one antibiotic is a macrolide antibiotic and another antibiotic is selected from tetracyclines and quinolones.

5. A method according to claim 1 comprising administration of azithromycin, rifampicin and a third antibiotic selected from doxycycline and the quinolones.

6. A method according to claim 1 further comprising the administration of one or more further agents selected from other medications used in the management of coronary and other vascular disease, other medications that enhance host defence mechanisms important in the eradication of intracellular pathogens, selective and non-selective cyclooxygenase inhibitors; other antiplatelet drugs; betablockers; antiarrhythmics; calcium channel blockers; other anticoagulant drugs; nitrate medicines and HMG-Coareductase inhibitors; immune response modifiers selected from cytokines; colony stimulating factors; tumour necrosis factors alpha and beta; interferon alpha, beta and gamma; peptides which bind to macrophage and lymphocyte surface receptors; glycoproteins which mimic cytokines; and other mediator molecules; prednisone and related steroids, azathioprine, mofetil mycofenolate and related purine antagonists, cyclophosphamide and related alkylating agents, methotrexate and related folate antagonists, thalidomide, chloroquine and related antimalarial compounds, levamisole, cyclosporin A, rapamycin and FK506.

7. A method according to any one of claims 1-6 wherein said condition is selected from atherosclerotic vascular disease affecting coronary arteries, aorta, carotid arteries and other arteries, renovascular and glomerular disease, aortic vascular disease,

peripheral vascular disease, carotid and cerebrovascular disease, atrial fibrillation and other cardiac arrhythmias, myocardial infarction, unstable and stable angina, valvular heart disease, cardiomyopathy, myocarditis and vasculitis, upper and lower respiratory tract infection, pneumonia, asthma, chronic airflow limitation, sarcoidosis, lung cancer, granulomatous hepatitis, dementia and gynaecologic and urologic mucosal infections, clinical syndromes that result from infection by mycoplasma, Bartonella, Leptospirosis and Q fever.

8. A method according to any one of claims 1-6 for the treatment or prevention of a condition associated with *Chlamydia pneumoniae* infection.

9. Use of at least two different antibiotics or antimicrobial agents selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones for the manufacture of a medicament for the treatment or prevention of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment.

10. Use of three, four or more different antibiotics or antimicrobial agents selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones for the manufacture of a medicament for the treatment or prevention of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment.

11. At least two different antibiotics or antimicrobial agents selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones, when used for the treatment or prevention of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment.

12. A pharmaceutical composition for the treatment of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment, the composition comprising at least two different antibiotics or antimicrobial agentsselected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones.

13. A pharmaceutical composition according to claim 12 which further includes a third, or a third and a fourth, or a third, a fourth and one or more further different antimicrobial agents or antibiotics.

14. A pharmaceutical composition according to claim 12 wherein one antibiotic is a macrolide antibiotic and another antibiotic is selected from tetracyclines and quinolones.

15. A pharmaceutical composition according to claim 12 comprising at least three different antibiotics, each antibiotic being from a different class, selected from tetracyclines, macrolides, quinolones, chloramphenicol, sulfonamides, co-trimoxazole and oxazolidinones.

16. A pharmaceutical composition for the treatment of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment, the composition comprising at least two different antibiotics or antimicrobial agents selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, sulfonamides, co-trimoxazole and oxazolidinones and not including a rifamycin.

17. A pharmaceutical composition for the treatment of a condition associated with infection by *Chlamydia* species or similar susceptible microorganisms in a patient in need of such treatment, the composition comprising a first antibiotic or antimicrobial agent selected from azithromycin, clarithromycin and roxithromycin, said second antibiotic or antimicrobial agent which is rifampicin, and a third antibiotic or antimicrobial agent selected from doxycycline and ofloxacin.

18. A pharmaceutical composition according to any one of claims 12, 16 or 17 further comprising one or more other medications used in the management of coronary and other vascular disease.

19. A pharmaceutical composition according to any one of claims 12, 16 or 17 further comprising one or more other medications that enhance host defence mechanisms important in the eradication of intracellular pathogens.

20. A pharmaceutical composition according to any one of claims 12, 16 or 17 further comprising one or more additional agents selected from selective and non-selective cyclooxygenase inhibitors; other antiplatelet drugs; betablockers; antiarrhythmics; calcium channel blockers; other anticoagulant drugs; nitrate medicines and HMG-Coareductase inhibitors.

21. A pharmaceutical composition according to any one of claims 12, 16 or 17 further comprising one or more immune response modifiers selected from cytokines; colony stimulating factors; tumour necrosis factors alpha and beta; interferon alpha, beta and gamma; peptides which bind to macrophage and lymphocyte surface receptors; glycoproteins which mimic cytokines; and other mediator molecules.

22. A pharmaceutical composition according to any one of claims 12, 16 or 17 further comprising one or more drugs with immunosuppressive activity selected from prednisone and related steroids, azathioprine, mofetil mycofenolate and related purine antagonists, cyclophosphamide and related alkylating agents, methotrexate and related folate antagonists, thalidomide, chloroquine and related antimalarial compounds, levamisole, cyclosporin A, rapamycin and FK506.

23. A pharmaceutical composition according to any one of claims 12, 16 or 17, wherein said antimicrobial agents are formulated within a single capsule or tablet.

24. A pharmaceutical composition according to any one of claims 12, 16 or 17, wherein said antibiotics or antimicrobial agents are microencapsulated.

25. A pharmaceutical composition according to claim 24 wherein said microencapsulation is adapted to cause said antibiotics or antimicrobial agents to be released differentially in different regions of the gastrointestinal tract.

26. A pharmaceutical composition comprising a first antibiotic or antimicrobial agent provided with a first pharmaceutically acceptable coating and at least a second antibiotic or antimicrobial agent optionally provided with a second pharmaceutically acceptable coating, said antibiotics or antimicrobial agents being selected from the group consisting of tetracyclines, macrolides, quinolones, chloramphenicol, rifamycins, sulfonamides, co-trimoxazole and oxazolidinones.

27. A pharmaceutical composition according to claim 26, wherein said coating or coatings are adapted to cause said first and second antibiotics or antimicrobial agents to be released in first and second environments in the gastrointestinal tract of a patient to whom said composition is administered, wherein an effective amount of said first antibiotic or antimicrobial agent is capable of being absorbed into the bloodstream of said patient from said first environment and an effective amount of said second antibiotic or antimicrobial agent is capable of being absorbed into the bloodstream of said patient from said second environment.

28. A pharmaceutical composition according to claim 27 further comprising a third antibiotic or antimicrobial agent provided with a third pharmaceutically acceptable coating, said third coating being adapted to cause said third antibiotic or antimicrobial agent to be released in a third environment in the gastrointestinal tract of a patient to whom said composition is administered, wherein an effective amount of said third antibiotic or antimicrobial agent is capable of being absorbed into the bloodstream of said patient from said third environment.

29. A pharmaceutical composition according to claim 28 wherein said first antibiotic or antimicrobial agent is a macrolide, said second antibiotic or antimicrobial agent is a rifamycin, and said third antibiotic or antimicrobial agent is a tetracycline or a quinolone.

30. A pharmaceutical composition according to claim 29 wherein said first antibiotic or antimicrobial agent is selected from azithromycin, clarithromycin and roxithromycin, said second antibiotic or antimicrobial agent is rifampicin, and said third antibiotic or antimicrobial agent is selected from doxycycline and ofloxacin.

31. A pharmaceutical composition according to claim 26 wherein said coatings are applied by microencapsulation.

32. A pharmaceutical composition according to claim 27 wherein said first and second environments are different.

33. A pharmaceutical composition according to claim 28 wherein said first, second and third environments are different.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00528

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 31/015, 31/16, 31/165, 31/42, 31/47, 31/35

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K 31/015, 31/16, 31/165, 31/42, 31/47, 31/35

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT: TETRACYCLIN:, MACROLID:, QUINOLON:, CHLOREMPHENICOL:, SULFONAMID:, COTRIMOXAZOL:, OXAZOLIDON:, CHLAMYDIA
CAPLUS: TETRACYCLIN, MACROLD, QUINOLON, SULFONAMIDE, COTRIMAXAZOL, OXAZOLIDONE, CHLOREMPHENICOL, CHLAMYDIA.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Bedos JP, Presse Medicale (26 September 1998) 27(28), 1440-1 Summary	1-33
X	Pichichero ME, Annals of Emergency Medicine (March 1995) 25(3), 390-403, See page 397, last line column 1-lines 1-3, column 2	1-33
X	Bowie WR, Infection (1982) 10 Suppl 1, S46-S52, see page S50, lines 16-21, column 2	1-7, 9-33

[X] Further documents are listed in the continuation of Box C

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	18 AUG 1999
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer **S. CHANDRA** Telephone No.: (02) 6283 2264

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00528

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Bowie WR et al., Lancet (December 1976) 2 (7998), 1276-8, see page 1278, lines 12-13, column 1	1-7, 9-33
X	Bowie WR et al., Drugs (May 1984) 27(5) 459-68, see abstract	1-7, 9-33
X	GB 1463550 (BODRERO G) 2 February 1977	12-33
X	WO 96/33670 (UNIV TEXAS SYSTEM) 31 October 1996	12-33
X	Patent Abstract of Japan, C311, page 82, JP-60120815, A (TOYO JOZO KK) 28 June 1985	12-33

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU 99/00528

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	96/33670	AU	55756/96	CA	2219342	EP	830107
		US	5624704	US	5902283		
JP	60120815	DE	3443632	US	4743591		

END OF ANNEX

WO 01/76585
PCT/EP01/03709

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

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NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
ZAMBON GROUP S.P.A.
Industrial Property & Intelligence
Via Lillo del Duca, 10
I-20091 Bresso
ITALIE

Date of mailing (day/month/year) 18 October 2001 (18.10.01)	
Applicant's or agent's file reference IT-368	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/EP01/03709	02 April 2001 (02.04.01)	11 April 2000 (11.04.00)

Applicant
ZAMBON GROUP S.P.A. et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
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3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   18 October 2001 (18.10.01) under No. WO 01/76585

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

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(54) Title: USE OF THIAMPHENICOL AND DERIVATIVES THEREOF FOR THE PREPARATION OF PHARMACEUTICAL COMPOSITIONS USEFUL IN THE TREATMENT OF *CHLAMYDIA PNEUMONIAE* INFECTIONS

(57) Abstract: Use of thiamphenicol and derivatives thereof for the preparation of pharmaceutical compositions useful for the treatment of *Chlamydia pneumoniae* infections is described.

Antimicrobial Activity of Thiamphenicol-glycinate-acetylcysteinate and Other Drugs against *Chlamydia pneumoniae*

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Summary

Chlamydia pneumoniae is responsible for respiratory tract infections of both upper and lower respiratory tract. Although this bacterium is one of the most widespread pathogens of man, there are limited data on the antibiotic treatment of *C. pneumoniae* infections. The aim of this study has been to evaluate the in vitro activity of thiamphenicol glycinate acetylcysteinate (TGA, CAS 20192-91-0) in comparison with molecules with established activity against *C. pneumoniae*, as well as macrolides and quinolones.

The results have shown that TGA and clarithromycin (CAS 81103-11-9) are the most active drugs tested, but it is important to underline that the minimal inhibitory concentration (MIC) ranges of TGA are very much lower than the breakpoint of thiamphenicol for the respiratory pathogens. In conclusion, the good antimicrobial in vitro activity of TGA against *C. pneumoniae* together with its in vivo characteristics, in particular the high concentration reached in lung and the combination with the mucolytic agent N-acetylcysteine (NAC, CAS 616-91-1), can make a valid choice in the treatment of respiratory tract infections caused by *C. pneumoniae*. These findings need further evaluation by clinical studies.

Zusammenfassung

Antimikrobielle Aktivität von Thiamphenicol-Glycinat-Acetylcysteinat und anderen Arzneistoffen gegen *Chlamydia pneumoniae*

Chlamydia pneumoniae verursacht Atemwegsinfektionen der oberen und unteren Atemwege. Obwohl dieses Bakterium ein weit verbreiteter Erreger beim Menschen ist, sind nur wenige Studien hinsichtlich einer Behandlung bei einer *C. pneumoniae*-Infektion verfügbar. Das Ziel der vorliegenden Studie war eine Evaluierung der In-vitro-Aktivität von Thiamphenicol-Glycinat-Acetylcysteinat (TGA, CAS 20192-91-0) im Vergleich zu Arzneistoffen mit bekannter Wirksamkeit gegen *C. pneumoniae* sowie Makroliden und Chinolonen. Die Ergebnisse zeigen, daß TGA und Clarithromycin (CAS 81130-11-9) unter den getesteten Substanzen die größte Wirksamkeit aufweisen. Allerdings lagen die durchschnittlichen minimalen Hemmkonzentrationen von TGA gegen *C. pneumoniae* deutlich unter denen von Thiamphenicol. Als Folge der guten antimikrobiellen In-vitro-Aktivität von TGA gegen *C. pneumoniae* zusammen mit den In-vivo-Charakteristika, insbesondere der hohen Gewebekonzentration in der Lunge, stellt die Substanz in Kombination mit dem schleimlösenden Medikament N-Acetylcystein (NAC, CAS 616-91-1) möglicherweise eine gute Wahl bei der Behandlung von durch *C. pneumoniae* verursachten Atemwegserkrankungen dar. Diese Ergebnisse bedürfen einer weiterführenden klinischen Objektivierung.

Key words

- Antimicrobial susceptibility
- CAS 81103-11-9
- Chlamydia pneumoniae
- Chl ramphenicol
- Respiratory tract infecti ns
- Thiamph nicol glycinate acetylcysteinate

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1. Introduction

Chlamydia pneumoniae is an intracellular bacterium responsible for respiratory tract infections of both upper and lower respiratory tract. This bacterium is a widespread pathogen of man and, primary infections are documented in children from 5 to 14 years by the rising prevalence of IgG anti-*C. pneumoniae* antibodies starting at school age and reaching a prevalence of 50–60 % positivity at the second decade of life [1, 2]. In children the infection is generally mild or asymptomatic, but it may be more severe in elderly people.

C. pneumoniae is responsible for approximately 10 % of cases of atypical pneumonia and 5 % of cases of bronchitis. It is also associated with reactive airway disease in children and new-onset asthma and asthmatic bronchitis in adults [3, 4]. Sinusitis caused by *C. pneumoniae* alone or associated with infection of the lower respiratory tract has been described and, moreover, *C. pneumoniae* has been isolated from middle ear fluids of patients with otitis media with effusion [5].

Antibiotic treatment may require a prolonged period and, cases of chronic persistent *C. pneumoniae* infection in which antibiotic therapy failed to eradicate the organism have also been reported.

Limited data on the treatment of *C. pneumoniae* pulmonary infections are available, suggesting that treatment with either erythromycin or doxycycline may not be always effective.

Azithromycin (AZM, CAS 83905-01-5) and clarithromycin (CLR, CAS 81103-11-9) have attracted interest as potential therapeutical agents for the treatment of respiratory tract infection (RTI) because they show high in vitro activity against *C. pneumoniae* and other agents involved in these infections [6].

The quinolone antibiotics are also potential therapeutical agents for *C. pneumoniae* infections; the levo isomer appears to be more active than the dextro isomer [7].

In spite of the necessity of antibiotic susceptibility data, in vitro studies are limited due to the low number of clinical isolates available and, moreover, the difficulty to obtain a high titre of *C. pneumoniae*; in fact, in vitro passages of fresh isolates are often unsuccessful.

Thiamphenicol glycinate acetylcysteinate (TGA, CAS 20192-91-0) is a derivative of Thiamphenicol (CAS 15318-45-3), a broad-spectrum antibiotic in which the water soluble glycine ester has been salified with acetylcysteine. TGA, a single chemical entity, keeps the pharmacological properties of the individual constituents: the antibiotic action of thiamphenicol and the mucolytic activity of acetylcysteine. These activities could act synergically in the therapy of airways infections [8, 9].

In fact, TGA shows a good in vitro antimicrobial activity against respiratory pathogens such as *Streptococcus pneumoniae, Streptococcus pyogenes, Moraxella catharralis,* and *Haemophilus influenzae.* The in vitro efficacy is also supported by the mucolytic activity of NAC.

As indicated by many studies, TGA is a compound which, upon administration, is effectively available at various sites of infection since it is not inactivated by the metabolic route nor is it bound to the plasma proteins. It freely diffuses into the interstitial fluid and into the cells of the different tissues.

In fact, TGA, when reaching the tissue, penetrates deeply providing high tissue levels which are effective against certain localized infections, and do not simply produce a superficial antibacterial effect.

As concern the pulmonary infections, thiamphenicol, especially when combined with N-acetylcysteine, is particularly indicated in the treatment of these infections. As demonstrated in our previous study, NAC seems to facilitate antimicrobial drug penetration into the pulmonary tissue. The lung/serum ratio found in this study was very high (after 12 h from administration the ratio was 5) indicating an optimal penetration into the lung tissue [10].

The antimicrobial activity of chloramphenicol on Chlamydia species is well documented and widely demonstrated by growth inhibition in tissue cultures. But no data are available on thiamphenicol activity against Chlamydia species, except for its activity in comparison to chloramphenicol on the outcome of *Chlamydia psittaci* infection in the chick embryo [11].

Thiamphenicol is a chemical analogue of chloramphenicol with a methylsulphonyl group replacing the nitro group in the para position of the aromatic ring. The p-nitro group of chloramphenicol has been shown to play a central role in the pathogenesis of aplastic anaemia. The absence of a p-nitro group in thiamphenicol is therefore advanced as strong evidence that thiamphenicol may not induce aplastic anaemia [12].

These properties together with the favourable pharmacokinetic profile prompted us to evaluate the in vitro antimicrobial activity of TGA against recent isolates of *C. pneumoniae.*

The present study compared the in vitro activity of TGA, CLR, AZM, amoxicillin (AML, CAS 26787-78-0), doxycycline (DO, CAS 17086-28-1), ciprofloxacin (CIP, CAS 85721-33-1), ceftriaxone (CRO, CAS 104376-79-6) and tetracycline (TE, CAS 60-50-8) against 9 clinical isolates of *C. pneumoniae* and 2 reference strains belonging to public type culture collections.

2. Materials and methods

2.1. C. pneumoniae strains

C. pneumoniae ATCC VR 2282 (TWAR TW-183) and *C. pneumoniae* ATCC VR 1356 (TWAR 2023) were obtained from the American Type Culture Collection. The other 9 strains tested are clinical isolates obtained in the period between 1997 and 1999.

2.2. Antibiotics

The antibiotics used were: TGA, CLR, AZM, AML, DO, CIP, CRO, and TE, all from the respective manufacturers.

The antibiotics were dissolved according to the instructions of the manufacturer.

2.3. Cell cultures

Monolayers of Hep-2 cells were prepared by seeding 2×10^5 cells/ml in Eagle's minimal essential medium (MEM) with 10 % foetal calf serum supplemented with L-glutamine, on 12 mm cover slips and left at 35 °C, 5 % CO_2 for 24 to 48 h for confluent growth. The growth medium was removed and Hep-2 monolayers were inoculated with the different strains at predetermined concentration calculated to give $3–5 \times 10^2$ inclusions per well.

The monolayers, inoculated with *C. pneumoniae*, were centrifuged at 1700 g for 60 min at 30 °C.

Supernatant was removed and was replaced by 2.0 ml of MEM containing 2 % foetal calf serum, L-glutamine, cycloheximide (1 µg/ml), and serial two fold dilutions of the respective drug.

In positive control, no antibiotics were added and negative controls were considered wells not inoculated with *C. pneumoniae*.

Cells were incubated at 35 °C in atmosphere additionated with 5 % CO_2 for 3 days. Then the monolayers were fixed with acetone for 10 min at –20 °C and stained with a fluorescein-conjugated antibody specific for *C. pneumoniae* (Argene Biosoft, Varilhes, France) and observed under a fluorescence microscope.

All tests were run in duplicate. The number of inclusions was counted and the MIC, the lowest concentration at which complete inhibition of inclusion formation was observed, was determined.

3. Results

The activities of the drugs tested are shown in Table 1. CLR and TGA showed to be the most active drugs tested in this in vitro study with a MIC range from 0.03 to 0.25 µg/ml for both drugs. A good antimicrobial activity was also observed with AZM, TE (MIC range 0.06–0.5 µg/ml for both the drugs) and DO (MIC range 0.06–0.25 µg/ml). CIP was less active (MIC range 0.5–2 µg/ml) and, moreover, all the strains of *C. pneumoniae* tested were not susceptible in vitro to AML and CRO (MIC > 16 µg/ml).

In Table 2 the MIC values obtained in the two different test runs are shown.

Table 1: Antimicrobial in vitro activity against *C pneumoniae* isolates.

Antibiotics	MIC range (µg/ml)
Clarithromycin	0.03–0.25
Azithromycin	0.06–0.5
Amoxicillin	>16
Doxycycline	0.06–0.25
Ciprofloxcacin	0.5–2
Ceftriaxone	>16
Tetracycline	0.06–0.5
Thiamphenicol-glycinate-acetylcysteinate	0.03–0.25

Table 2: Minimal inhibitory concentrations (µg/ml) of selected antibiotics against *C. pneumoniae* strains for two different test runs.

Isolate	Run	CLR	AZM	DO	CIP	TE	TGA
1	I	0.03	0.5	0.06	1	0.06	0.03
	II	0.03	0.125	0.125	1	0.06	0.06
2	I	0.25	0.06	0.125	0.5	0.06	0.03
	II	0.03	0.25	0.125	2	0.06	0.03
3	I	0.03	0.06	0.125	0.5	0.125	0.125
	II	0.03	0.06	0.125	1	0.125	0.125
4	I	0.125	0.5	0.06	1	0.125	0.25
	II	0.06	0.5	0.06	1	0.125	0.06
5	I	0.03	0.25	0.125	0.5	0.125	0.125
	II	0.03	0.25	0.06	0.5	0.25	0.125
6	I	0.03	0.125	0.06	2	0.06	0.03
	II	0.125	0.25	0.06	2	0.06	0.03
7	I	0.125	0.25	0.06	2	0.125	0.03
	II	0.125	0.25	0.06	1	0.125	0.03
8	I	0.125	0.25	0.25	0.5	0.06	0.125
	II	0.125	0.25	0.06	2	0.06	0.03
9	I	0.125	0.5	0.25	0.5	0.06	0.125
	II	0.03	0.125	0.25	0.5	0.5	0.125
ATCC	I	0.03	0.06	0.06	0.5	0.06	0.03
VR 1356	II	0.03	0.125	0.06	0.5	0.125	0.03
ATCC	I	0.03	0.125	0.125	0.5	0.06	0.06
VR 2282	II	0.125	0.25	0.25	1	0.06	0.03

MIC values for amoxicillin and ceftriaxone were > 16 µg/ml. I: first test run; II: second test run; CLR: clarithromycin; AZM: azithromycin; DO: doxycycline; CIP: ciprofloxacin; TE: tetracycline.

4. Discussion

Data on in vitro susceptibility of *C. pneumoniae* are limited and the results reported in literature are variable because the method is not completely standardized.

The different cell lines inoculated, or the treatment of monolayer, the inoculum size and the timing of drug's addition are all parameters that may influence the results.

Moreover in different tests, run with the same method, we found an intra-strain and strain-to-strain variation in our antibiotic susceptibility results.

The results obtained in the present study are in general consistent with those reported by other authors [13, 14].

The novelty of this study is that it represents the first work on the in vitro activity of TGA against *C. pneumoniae*.

An interesting item of the results was the MIC value. The MIC ranges, were not only much lower than the break-point of thiamphenicol for the respiratory germs, but they were comparable to those obtained with molecules with established activity against *C. pneumoniae*, as well as macrolides and quinolones. It is evident that the in vitro data are not enough for a safe therapeutic use, but they become surely important when they are associated to the pharmacokinetic and bioavailability properties, as well as the mode of administration and the compliance of the patient.

The results obtained with clarithromycin agree with those reported by other authors who also underline the

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good tissue and intracellular penetration of this antimicrobial agent [15]. Nevertheless, this drug needs a prolonged therapy with a multiple daily dosing.

In the present study, azithromycin, doxycycline and tetracycline also showed in vitro activity against all isolates of *C. pneumoniae*. This is in accordance with other authors, who reported these drugs to be active against *C. pneumoniae* at concentrations lower than the serum levels found during antibiotic therapy [16].

Considering the in vivo characteristics, azithromycin has a better intracellular penetration into alveolar macrophages and into bronchial epithelia compared with doxycycline and tetracycline [17].

Tetracycline has a good activity against *C. pneumoniae*, being largely used for the treatment of Chlamydia associated infections. Actually, doxycicline shows the same in vitro efficacy and has replaced tetracycline because of the better patient compliance [18].

In conclusion, to suggest antibiotic therapy in RTI's the in vitro susceptibility data have to be considered together with information about the tissue penetration, the compliance and the intracellular penetration, especially into bronchial epithelia and alveolar macrophages.

For these reasons, the good antimicrobial in vitro activity of TGA against *C. pneumoniae* together with its in vivo characteristics, such as high tissue concentrations in the lung, the combination with the mucolytic agent NAC that improves the activity of thiamphenicol in the bronchial tree through the reduction of mucous viscosity facilitating the expectoration [19], and its tolerability, make it promising drug for the treatment of RTI's caused by *C. pneumoniae*.

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Clinical features of *Chlamydia pneumoniae* acute respiratory infection

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Chlamydia pneumoniae is a worldwide respiratory pathogen involved in 6–20% of community-acquired pneumonias and in about 5% of acute exacerbations of chronic bronchitis. Preliminary data also indicate a possible association between *Chlamydia pneumoniae* infection and asthma. Further studies are needed to elucidate whether *Chlamydia pneumoniae* is merely a precipitant of asthma symptoms or is actually one of the causes of asthma.

Key words: *Chlamydia pneumoniae*, community-acquired pneumonia, asthma

Chlamydia pneumoniae has recently been recognized as a cause of respiratory tract infections [1,2]. It has been classified as a third species of the *Chlamydia* genus by means of ultrastructural and DNA homology analysis [3].

C. pneumoniae is an obligate intracellular, Gram-negative bacterium involved in a wide spectrum of respiratory tract infections of the upper respiratory tract (pharyngitis, sinusitis and otitis) and lower respiratory tract (acute bronchitis, exacerbation of chronic bronchitis and asthma, and community-acquired pneumonias) in both immunocompetent and immunocompromised subjects [4–21]. Several studies have recently stressed the importance of this agent in the development of respiratory diseases, showing a high incidence and prevalence of infections worldwide. Specific antibody prevalence in Western countries is low in pre-school children and climbs to over 50% in adults, remaining high in old age due to *C. pneumoniae* reinfection among adults [7].

In this paper the clinical characteristics of *C. pneumoniae* acute infection will be discussed, focusing on the possible pathogenetic role of this agent in asthma.

DISEASES ASSOCIATED WITH ACUTE *C. PNEUMONIAE* INFECTION

C. pneumoniae seems to be an important cause of human respiratory tract disease. Several reports show a high incidence of infection in community-acquired pneumonia, ranging from 6% to 25%, and a remarkable role in pneumonia outbreaks in closed communities like military garrisons, schools and families [8,12–16]. Further, *C. pneumoniae* is involved in upper respiratory tract infections (pharyngitis, sinusitis, otitis), acute bronchitis and exacerbations of chronic bronchitis [5,6]. Recently, Hahn et al [10] reported a possible etiopathogenetic role of this pathogen in adult onset of asthma and in asthma exacerbation.

Several other diseases associated with *C. pneumoniae* infection, such as erythema nodosum, Guillain-Barré syndrome, culture-negative endocarditis, thyroiditis, arthritis and encephalitis, have been sporadically reported [22,24]. Seroepidemiologic evidence of a possible association between *C. pneumoniae* infection and sarcoidosis has also been suggested [25]. Saikku et al [26,27] and other authors [28–32] also found an association between *C. pneumoniae* and coronary artery disease. Table 1 summarizes published data on the incidence of *C. pneumoniae* infections.

Table 1 Incidence of *Chlamydia pneumoniae* infections

Disease	Range of incidence
Asymptomatic infection	Common
Flu-like syndrome	Common
Community-acquired pneumonia	6–25%
Outbreaks in closed communities	–
Family outbreaks	–
Upper respiratory tract infections	5–10%
COPD exacerbations	4–5%
Asthma attacks	1–18%

UPPER RESPIRATORY TRACT INFECTION

C. pneumoniae is involved in the etiology of acute pharyngitis, otitis and sinusitis with an incidence ranging from 5% to 10% [32,33]. In these diseases, no specific clinical manifestations have been shown. However, acute pharyngitis could be relatively severe with hoarseness, and one-third of patients present exudates with or without fever. Upper respiratory tract infections often herald or are associated with infections of the lower respiratory tract.

LOWER RESPIRATORY TRACT INFECTION (LRTI)

C. pneumoniae is increasingly reported as an important etiologic agent of LRTI. About 5% of COPD exacerbations are sustained by *C. pneumoniae*. Possible chronic *C. pneumoniae* infection in chronic bronchitis was suggested by Blasi et al [6], who found a significantly higher frequency of IgG anti-*Chlamydia pneumoniae* antibody, exceeding 6.3%, in patients with exacerbations of COPD compared to controls. This remarkably high prevalence could be due to either chronic infection by *C. pneumoniae*, as suggested by the increase of specific IgG prevalence and geometric mean titer with age, or a higher rate of acute infection in such patients. Von Hertzen et al [34] found no differences in serum IgG antibodies between patients and controls, but the difference in serum IgA prevalence was significant. Moreover, local sputum IgA antibodies against *C. pneumoniae*, absent in the majority of pneumonia patients, were a common finding in the sputa of chronic bronchitis patients, along with local antibodies against *Haemophilus influenzae* and *Branhamella catarrhalis*. *C. pneumoniae* might therefore be added to these bacteria, which have invariably been associated with the pathogenesis of chronic bronchitis.

Most reports rank this agent as being among the three most common etiologic agents of community-acquired pneumonia, generally presenting a mild, and in some cases self-limiting, clinical course [35,36], although sporadic cases of severe pneumonia have been reported [36,37]. The radiographic pattern of pneumonia is extremely variable, with a reported high incidence of sub-segmental consolidation [38,39].

Kleemola et al reported a seroepidemiologic study showing pneumonia epidemics in military garrisons sustained by *C. pneumoniae*, each epidemic lasting more than 6 months with an infective rate of about 80 per 1000 [8].

Family transmission, child to child, was described in Japan in 1990 [13]. Mordhorst et al [14] described an outbreak of *C. pneumoniae* infections in four farm families living close together in Denmark, with an unusually high incidence of symptomatic infections, particularly lower respiratory tract infections, among family members. These data support the human-to-human contact spread of *C. pneumoniae* infection, and point to the role of this agent in family cluster respiratory infections, although Aldous et al [15], in a serologic study of family serum samples conducted in 1966–1969, reported that acute infections more often affected a single family member than multiple members.

Blasi et al recorded a high rate (75%) of infection in two family outbreaks [16]. These data are in contrast to the low incidence of infection recorded during epidemics in military trainees in Finland [8] and in the serologic study of Aldous et al [15], while they are consistent with those reported by Mordhorst et al [14], who observed a family cluster with relatively high rate of infection.

In contrast to the numerous epidemiologic studies carried out in the general population, only limited data on *C. pneumoniae* infection in immunocompromised subjects are present in the literature [17–21]. Blasi et al have recently published preliminary results on *C. pneumoniae* seroprevalence in HIV-1-infected intravenous drug users and HIV-1 vertically infected children [17]. The seroprevalence of *C. pneumoniae* in the HIV-1-infected intravenous drug users (IDUs) was significantly higher than in both HIV-1-negative IDUs and immunocompetent subjects matched for age and sex. Recent reports suggest a possible role of *C. pneumoniae* as an etiologic agent of pneumonia in HIV-1-positive subjects [18–21]. In a retrospective serologic study, Blasi et al [21] observed an outbreak of *C. pneumoniae* infection in an ex-injection drug user community, where almost 25% of the residents were HIV-1 positive. The epidemic occurred in a small group of 26 subjects living and working in the same place. Their rate of HIV-1 positivity was remarkably high (50%). A higher *C. pneumoniae* infection rate in HIV-1-positive subjects was observed, with more than 76% of HIV-1-positive patients compared to only 38% of HIV-negative subjects suffering from acute *C. pneumoniae* infections. Moreover, most pneumonias occurred in HIV-1-positive patients. All lung infections were mild to moderate in severity, with a spontaneous recovery in one HIV-1-negative subject.

ASTHMA

Hyperresponsiveness is a key factor in the pathogenesis of asthma that might be determined by genetic factors and/or environmental exposures. Atopy, air pollution and smoke seem to be strongly associated with bronchial hyperresponsiveness. Moreover, familial

predisposition and/or genetic transmission of bronchial hyperresponsiveness have recently been observed [40,41]. In children, the role of viral infection in acute exacerbations of asthma has long been recognized, although its role in the pathogenesis of asthma is still controversial [42]. Less is known about the association between infections and asthma in adults. Commonly, influenza and the common cold precede asthma attacks, suggesting an etiopathogenic link between viral infection and acute exacerbation. This has been inferred from several epidemiologic studies, mainly conducted in the pediatric population [43,44].

The role of viruses as precipitants of asthma symptoms in adults seems to be less relevant than in children but epidemiologic data are conflicting. Beasley et al [45] reported an etiologic role of viruses in 10% of acute exacerbations of asthma, with a higher incidence (36%) in severe attacks. However, Sokhandan et al [46] did not find any evidence of viral infection in a small group of patients with acute asthma exacerbations. More recently, Nicholson et al [47] reported that 89% of patients with a cold had asthma symptoms. Moreover, the authors found that 44% of episodes with reductions in mean peak expiratory flow rate ≥50 l/min were associated with laboratory-confirmed infections, rhinoviruses and coronaviruses being predominant.

Bacterial infection seems to play a minor role in asthma attacks [48], although some evidence has drawn attention to *Mycoplasma pneumoniae* and recently to *C. pneumoniae* [10,49]. The latter plays an important aetiopathogenetic role in the development of acute respiratory tract infections [2,6] and Hahn et al [10] reported a possible association of *C. pneumoniae* infection with wheezing and adult-onset asthma. The results of this study showed a dose-response relationship between specific antibody titer level and prevalence of wheeze. Moreover, four out of 19 patients with acute *C. pneumoniae* infection subsequently developed asthma, and four others had exacerbation of previously diagnosed asthma.

Allegra et al [11] showed that acute exacerbation of asthma was associated with infection in 20% of their patients. Interestingly, viruses were involved in about 9% of asthma attacks, while acute infection with intracellular bacteria was detected in 11% of cases. Most of the latter (7/8 cases) were due to *C. pneumoniae* infection. Emre et al [50] reported the association of *C. pneumoniae* infection and reactive airway disease in children. The authors indicated that *C. pneumoniae* was a trigger of wheezing in asthmatic children and that treatment with macrolides, which are active against this agent, may improve the course of reactive airway disease in these patients.

Other findings also seem to indicate *C. pneumoniae* infection as a possible explanation for an increase of asthma in recent years [51]. For instance, cross-sectional data indicate an age-specific seroprevalence pattern [52] similar to the prevalence pattern of adult asthma [53]. *C. pneumoniae* infection preceding symptomatic adult asthma by about 10 years. Seroepidemiologic data from Finland [54] also show an increasing seroprevalence rate associated with increased asthma prevalence. Hahn and Golubjatnikov [55] reported a study on patients with adult-onset asthma in which 100% of patients were *C. pneumoniae* seroreactive.

C. pneumoniae has also been cultured from adult patients with acute exacerbation of asthma [56,57]. Moreover, Emre et al [50] identified the agent by culture in children with symptomatic asthma. In another study, Cunningham et al [58] showed that *C. pneumoniae* could be detected by polymerase chain reaction in a large proportion (47%) of children with asthma, both during exacerbations (24%) and when asymptomatic (27.7%). The latter study seems to support the hypothesis of a possible *C. pneumoniae* chronic infection in subjects with asthma.

Hahn has recently defined a '*Chlamydia*-asthma' hypothesis [59]. The hypothesis is based on the following evidence.

1. Production of *C. pneumoniae*-specific IgE and cytokines, along with a direct lesion of epithelial cells by the agent, leading to inhibition of ciliary motion [60] and a possible shedding of cells with an enhanced penetration of aeroallergens.
2. The capacity of *Chlamydia*, during reinfection or chronic infection, to produce T-cell-mediated immunopathologic diseases, with the possibility that *C. pneumoniae* could act as a long-term asthma promoter.
3. *C. pneumoniae* could infect vascular smooth muscle [61] and promote bronchial hyperreactivity via bronchial muscle infection.

Hence, the data available to date seem to support an etiologic link between *C. pneumoniae* infection and asthma, in both adults and children.

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